Review: Melanocyte Migration and Survival Controlled by SCF/c-kit Expression

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Melanocytes are derived from neural crest and migrate along the dorsolateral pathway to colonize the final destination in the skin. Stem cell factor and its receptor c-kit were identified as gene products of *Sl* and *W* mutant loci; both of them were known to have defects in melanocytes survival. In this review, we focus on the function of stem cell factor and c-kit in melanocyte migration and survival, which has become clearer in the last decade. By analysis of both molecules in wild-type and white spotting mutant mice, ligand and receptor set were shown to play multiple roles in the development of melanocytes in mouse ontogeny. Functional blockade of c-kit by specific monoclonal antibody illustrated distinct c-kit dependent and independent stages in melanocyte development. Finally, SCF transgene expression demonstrated that part of the c-kit dependent step is regulated by spatiotemporally specific ligand expression and also indicated the presence of c-kit independent melanocyte stem cells in postnatal skin. ***Keywords: SCF/c-kit/melanocyte/transgenic mouse/membrane-bound. Journal of Investigative Dermatology Symposium Proceedings 6:1–5, 2001***

Melanocytes (MC) originate from the neural crest and migrate along the dorsolateral pathway to colonize their final destination, the skin epidermal basal layer or hair follicles. From the various coat color pigmentation patterns found in many animal species, it was obvious that MC migration and/or survival in the periphery is affected by genetic or environmental differences during development. During the last decade, several molecules indispensable for MC development were identified as gene products encoded by coat color mutant loci. We would like to review the function of those molecules, with specific reference to stem cell factor (SCF) and its receptor c-kit in MC migration and survival.

MIGRATION AND DISTRIBUTION OF THE MC DURING MOUSE DEVELOPMENT

Due to the absence of useful MC markers, it was difficult to describe the migration of MC during mice embryogenesis. Jackson *et al* reported the specific expression of DOPAchrome tautomerase (DT) in a MC population and described the distribution pattern of MC in embryogenesis (Steel *et al*, 1992), and made a useful mouse model harboring the β-galactosidase gene under the control of the DT promoter region (Cable *et al*, 1995). Wehrle-Haller and Weston (1995) and our group (Kunisada *et al*, 1996; Yoshida *et al*, 1996a) independently detected MC distribution during development using c-kit as a specific marker for MC. As shown in **Fig 1**, the earliest MC population was detected in the dorsal region of the hindbrain at 9.5–10.0 dpc. Many MC were detected from the dorsal region from the hindbrain to the root of the tail bud by 10.5 dpc. MC gradually proliferate and migrate through the dermis horizontally to the ventral region, and then invade into the epidermis between 12.5 and 14.0 dpc. Epidermal MC also expand in number and migrate through the epidermal basal layer between 13.0 and 15.5 dpc (Yoshida *et al*, 1996a). This MC distribution pattern is clearly demonstrated in the coat color of Kit^{W}/+ or $Mitf^{MiWh}$/+, which show white spot formation in the belly and/or blaze, these are the last regions to be colonized by MC during development (see **Fig 1**, *top*, 13.5–14.5 dpc embryo). These results suggest that the number of MC in these mutant mice is smaller than with wild-type mice, though the migration pattern itself is basically unaffected.

THE MOLECULES AFFECTING MC DISTRIBUTION DURING EMBRYOGENESIS

Analysis of c-kit expression and MC distribution in the ''spotting'' mutant mice suggested the function of the molecules encoded at these mutant loci. MC number in the lethal spotting ($edn3^{ls}/edn3^{ls}$; Hosoda *et al*, 1994) mutant is very small prior to entry into the epidermis, indicating that endothelin-3 is an indispensable factor for MC proliferation during dermal migration (Yoshida *et al*, 1996a). Transgenic rescue of this mutant also suggested that endothelin-3 plays a pivotal role around day 11 dpc to 12 dpc when MC migrate through the dermis (Shin *et al*, 1999). Coat color spotting patterns of this mutant resemble the distribution patterns of day 10 dpc embryos (**Fig 1**; Yoshida *et al*, 1996a).

In contrast, there are several unique mutants with patchy white spots that are different to the MC distribution patterns of the

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Abbreviations: MC, melanocyte; hK14, human cytokeratin 14; PDGF, platelet derived growth factor; SCF, stem cell factor; Tg, transgenic; MoAb, monoclonal antibody.

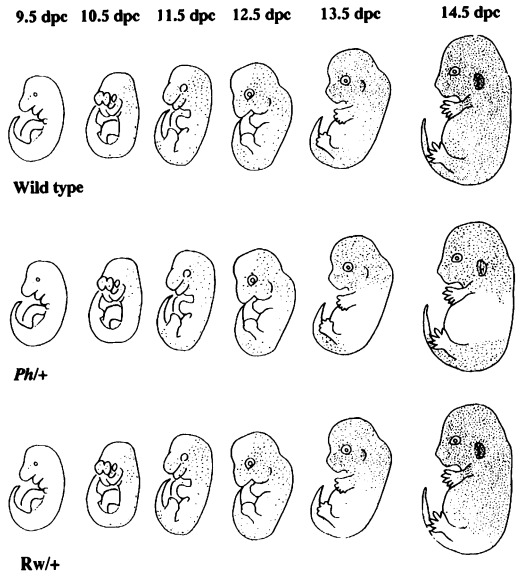

Figure 1. Migration and distribution of MC in developing mice. MC lineage cells were identified as bipolar c-kit-expressing cells in the developing mice skin by whole mount immunostaining. The upper panel shows the schematic MC distribution pattern of wild-type C57Bl/6 mice during development (adapted from Figure 1 of Yoshida *et al*, 1996a). The middle and bottom panels each show the MC distribution patterns of $Pdgfra^{Ph}$/+ and *Rw*/+ mutant mice.

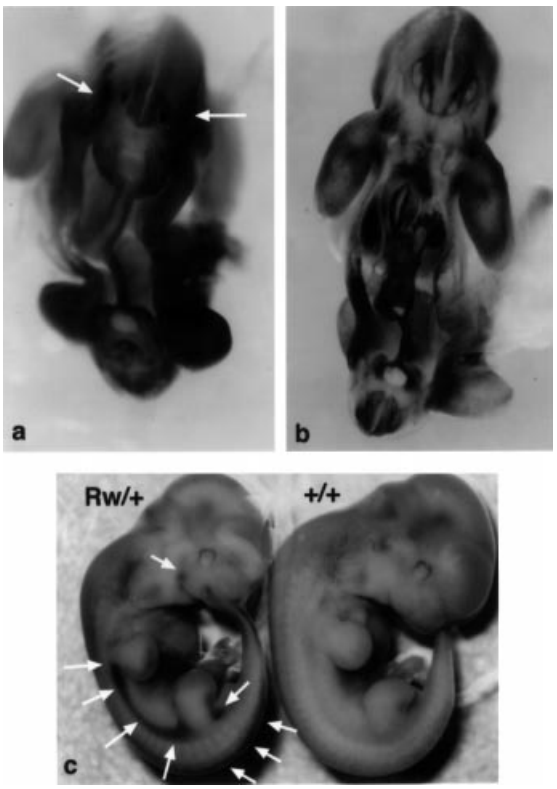

Figure 2. Expression of SCF and c-kit in 10.5 dpc wild-type embryo and c-kit expression in Rw/+ embryo. SCF is expressed in various organs, including the nervous system, heart, and gonad, and is intensively expressed in somites (*white arrows* in *a*) along which c-kit-expressing MC migrate. Note the contiguous expression of c-kit in multiple organs is detectable (*b*). In *Rw*/+ mutants, aberrant expression of c-kit in mesenchymal tissue was demonstrated by whole mount immunostaining (*white arrows* in *c*). Specimens were fixed and immunostained as described (Yoshida *et al*, 1996a; Nishimura *et al*, 1999), with commercially available antibodies (antimouse SCF polyclonal antibody; antimouse c-kit monoclonal antibody ACK45).

embryo. From genetic analysis, several independent coat color mutation loci, *W* (=*Kit*), *Ph* or *Rw*, were mapped to a tiny region on chromosome 5 (Silvers, 1979). *Ph*/+ mice have a large white spot covering the whole trunk. As the *Ph* mutation is a deletion including the PDGF receptor α gene, this mutant has several defects in the neural crest derived cell lineages (Stephenson *et al*, 1991; Morrison-Graham *et al*, 1992). The MC defect observed in the $Pdgfra^{Ph}$ mutant was thought at first to result from this receptor defect; however, pigmented MC were detectable in the $Pdgfra^{Ph}$/$Pdgfra^{Ph}$ mutant (Yoshida, unpublished observation). Furthermore, MC of wild-type mice do not express PDGF receptor α, and no effect on development was observed after the administration of a monoclonal antibody (MoAb) that blocks PDGF receptor α function (Takakura *et al*, 1996) or *in vitro* cultured MC (Yoshida, unpublished observation). The analysis of c-kit expression patterns in this mutant during development unmasked the mechanism of spotting formation. The MC distribution in Ph/+ mice at their early gestational stages is not different from those of wild-type mice (**Fig 1**, *middle*); however, aberrant expression of c-kit in the mesenchymal cells was found in unpigmented regions and MC gradually disappeared from this region (Wehrle-Haller *et al*, 1996). Ectopic expression of c-kit by mesenchymal cells may deplete local SCF resulting in ligand starvation-induced MC apoptosis. This idea was originally presented by Duttlinger *et al* (1993) in the analysis of the Kit^{Wsh}/+ mutant spotting pattern formation.

Like these mutant mice, *Rw*/+ mice also have a rump region specific white spot (Silvers, 1979), and this mutation also has the chromosomal rearrangement near the c-kit gene (Nagle *et al*, 1994). MC number and distribution pattern of *Rw*/+ mutants were comparable with the wild-type mice, but MC gradually disappeared from the mesenchymal region with aberrant expression of c-kit (**Fig 1**, *bottom*; **Fig 2*c***). As shown in **Fig 2(*a*)**, SCF is expressed in the dermo-myotome of 10–11 dpc embryo where c-kit-expressing MC migrate along to the periphery (**Fig 2*b***). Similar observations of MC migration in this mutant were reported recently (Jordan and Jackson, 2000). These findings suggested that the rump spotting of *Rw*/+ mutants resulted from a similar mechanism to Kit^{Wsh}/+ and $Pdgfra^{Ph}$/+ mutants.

In contrast, aberrant expression of SCF does not induce a white spot. Because of transcriptional defects, MGF^{Slcon}/MGF^{Slcon} mutant mice express SCF in some ectopic regions such as the reproductive tract, genitalia, lip, or nipples (Beechey and Searle, 1983). Those ectopic SCF expressions induce the migration, proliferation, and survival of MC outside of hair follicles or the epidermis (Bedell *et al*, 1995).

This mutant phenotype suggests that MC migration and survival is finely controlled by SCF and c-kit expression during embryogenesis.

ANALYSIS OF SCF AND C-KIT FUNCTION IN MC DEVELOPMENT BY FUNCTION BLOCKAGE

An epigenetic approach is required in order to investigate the function of SCF and c-kit in each MC developing step, as SCF and c-kit defective mutants die perinatally and their MC development is affected at an earlier stage. We have established the c-kit blocking MoAb ACK2 and analyzed MC development by injecting this antibody in to the wild-type mice at various stages of development (Nishikawa *et al*, 1991; Yoshida *et al*, 1993; Okura *et al*, 1995; Yoshida *et al*, 1996a). As the removal of SCF and the addition of ACK2 both induced apoptosis of MC (Okura *et al*, 1995; Ito *et al*, 1999), this strategy can establish the SCF defective state at any developmental stage. Using this MoAb injection technique, we found that SCF and c-kit interaction is required for (i) survival of MC during migration in the dermis, (ii) survival of MC in the epidermal sheet prior to entering the hair follicles, and (iii) survival

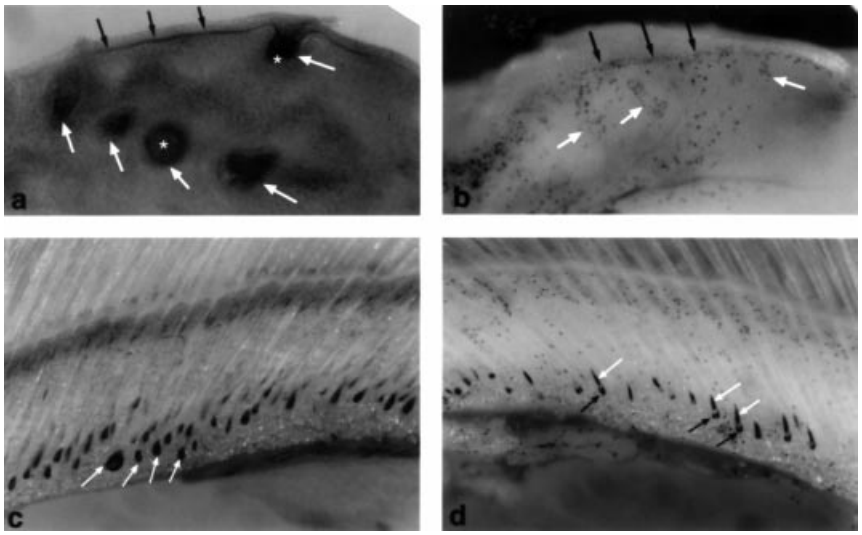

Figure 3. Expression of SCF and c-kit in 15.5 dpc embryo skin and day 8 neonatal skin. SCF is expressed in the 15.5 dpc epidermal basal layer (*black arrows*) and hair follicles (*white arrows* in *a*) where the c-kit expressing MC is also located (*black and white arrows* in *b*). Note that dermal papilla cells also express SCF (*white asterisk* in *a*). After birth, SCF expression gradually decreases in the upper portion of the hair follicles, and is finally restricted to the hair matrix (*c*). c-kit-expressing MC (*white arrows*) and a part of hair matrix keratinocytes (*black arrows*) are found in the vicinity of SCF-expressing hair matrix cells (*d*). Note the contiguous pattern of SCF and c-kit is also detectable in the dermal region where a weak level of SCF is expressed and many c-kit-expressing mast cells exist (compare *c* and *d*).

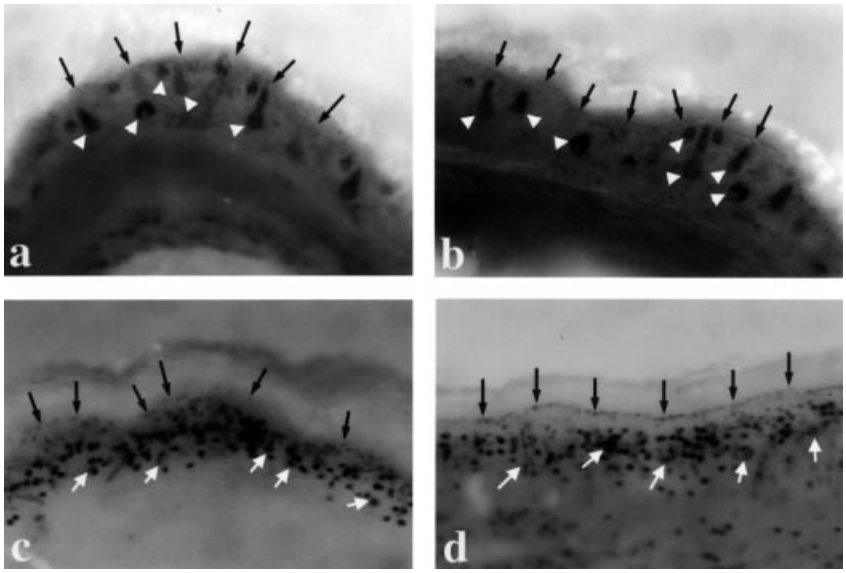

Figure 4. Expression of SCF and survival of MC in transgenic mouse epidermis. Expression of transgene product SCF protein was detectable in transgenic (*b*, *black arrows*) but not in wild-type mice epidermis (*a*, *black arrows* indicating the epidermal basal layer) at 18.5 dpc. Note that the endogenous SCF expression in hair follicles was comparable between Tg and wild-type mouse (*white arrowheads* in *a*, *b*). At this stage, there are a few epidermal MC detectable even in wild-type mouse epidermis (*white arrows* in *c*) but the whole epidermal basal layer of Tg mice is lined with MC (*white arrows* in *d*). The number and distribution patterns of follicular MC were comparable between Tg and wild-type mice.

of MC proliferating in the anagen hair follicles. These results indicate that MC have distinct SCF/c-kit dependent or independent developmental stages, in contrast to the continuous expression of c-kit on the MC surface during embryogenesis. It is interesting question as to what regulates the c-kit dependency of MC.

EXPRESSION PATTERNS OF SCF AND C-KIT IN THE SKIN REGION

There are several reports that describe the expression patterns of SCF and c-kit in numerous organs including the skin (Manova and Bachvarova, 1991; Motro *et al*, 1991; Besmer *et al*, 1993), but the signal:noise ratio of those studies is not satisfactory to detect distinct cells expressing SCF in skin tissues. We tried to detect the SCF protein expression using several antibodies by means of whole-mount immunostaining, and succeeded in detecting its expression in skin tissues. As shown in **Fig 3**(***a***), SCF is expressed in the epidermal basal layer and the whole hair follicle keratinocyte of 15 dpc mice. Compatible with the expression pattern of its ligand, c-kit immunoreactive MC are located in the epidermal basal layer and whole hair follicles at this stage (**Fig 3*b***). After birth, murine MC gradually disappear from the epidermal basal layer (Hirobe, 1984). In accordance with this observation, the expression of SCF in the interfollicular epidermal layer decreases after hair follicle formation, and is restricted to the hair matrix epidermis in juvenile mice skin (**Fig 3*c***), where the c-kit expressing MC proliferate and produce pigment granules (**Fig 3*d***). Although these results appeared to contradict our previous report demonstrating the ability of the SCF gene promoter region to express transgene in dermal papilla during embryogenesis (Yoshida *et al*, 1996b), low level SCF expression in embryonic dermal papilla was also detected by immunostaining (**Fig 3*a***, *asterisk*). The difference between these two studies suggests that the enhancer domain that regulates epidermal SCF expression resides outside of the 10 kbp that we have studied (Yoshida *et al*, 1996b). These results clearly indicate the importance of SCF and c-kit interaction in the whole process of MC migration and differentiation in mice ontogeny. Contiguous expression patterns of this ligand and the receptor are also observed in the other organs (**Fig 2*a***, ***b***), suggesting this ligand/receptor signal transduction is regulated by the local expression but not by systematic circulation of the soluble ligand (data not shown).

MC SURVIVAL IN DIFFERENT ENVIRONMENTS

In humans and mammals such as dogs, horses, etc., skin MC reside in both the hair follicle and the epidermal basal layer. In contrast, most laboratory mice have no epidermal MC in hairy skin. What mechanism regulates MC residency in distinct regions of the skin

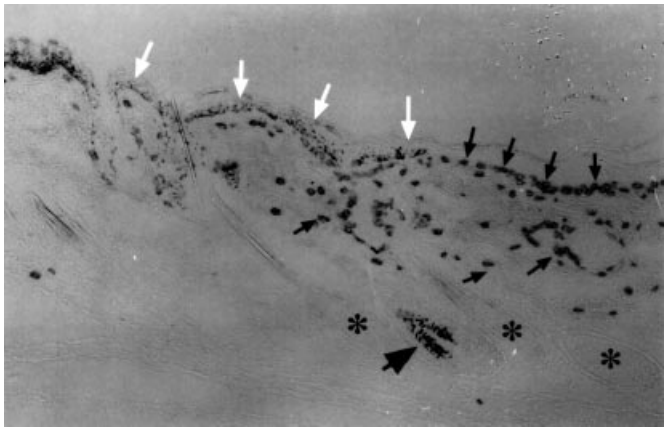

Figure 5. A pigmented hair follicle found in the pigmented skin recovering from c-kit function blockage in hK14-SCF transgenic mouse. In the pigmented region (*white arrows*) found in the transgenic mouse recovering from the neonatal injection of anti-c-kit MoAb, a few hair follicles with pigmented granules are detectable (*large black arrow*). Note the absence of pigment granules in the hair follicles in the vicinity (*asterisk*). *Small black arrows* detect the c-kit expression on epidermal MC and dermal mast cells.

between these species? Since epidermal and hair follicle MC express different cadherins on their surface (Nishimura *et al*, 1999), the difference may be detected in each MC population as an intrinsic mechanism. This suggested that the environment of human but not murine epidermal basal layers has the ability to support MC survival. To confirm this idea, we made a transgenic mice that expresses SCF under the control of the human cytokeratin 14 (hK14) gene promoter, which was reported to express transgene in the epidermal basal layer (Byrne *et al*, 1994). SCF transgene (Tg) expression allowed MC survival and development in the epidermal basal layer in mouse hairy skin (**Fig 4**; Kunisada *et al*, 1998a, b). Epidermal MC survival was dependent on SCF/c-kit interactions and was abolished by anti-c-kit MoAb injection. Local ligand expression therefore appears to regulate the requirement for c-kit in MC survival (Kunisada *et al*, 1998b).

C-KIT-DEPENDENT MC MIGRATION AND C-KIT-INDEPENDENT MC STEM CELL SURVIVAL

MC in the epidermis can proliferate and enter MC free regions during late gestational stages (Yoshida *et al*, 1996a). In addition, it appeared as if this proliferation was restricted by the contact inhibition between MC, as MC do not proliferate extensively in wild-type mice during late gestation, and the concentration in the fully MC filled region is constant during those stages (Yoshida, unpublished observation). These results supported the idea that MC can proliferate and expand their residing area if the appropriate conditions are established. This phenomenon was actually observed in hK14-SCF Tg mice. This transgenic mice epidermis can be depigmented by anti-c-kit antibody injection, but a few days later, tiny-pigmented spots are found that gradually expand in size and cover the whole skin with pigmentation (Kunisada *et al*, 1998b). This result suggests that even after birth, MC can migrate through the epidermal basal layer. This result also indicates that there must be a MC stem cell population that can survive independent of c-kit signal transduction. As there are always a single or a few pigmented hair follicles found in these tiny-pigmented spots (**Fig 5**), these c-kit-independent MC stem cells may reside in the hair follicles and migrate outside of the follicles in a c-kit-dependent manner. The location of the niche with the hair follicle where MC stem cells reside is an intriguing question.

FUNCTIONAL DIFFERENCE BETWEEN SOLUBLE AND MEMBRANE-BOUND FORMS OF SCF IN MC DEVELOPMENT

As mentioned above, SCF is expressed in tissues through which MC migrate. As the SCF *trans*-membrane region defective mutant Mgf^{Sld}/Mgf^{Sld} mice have no coat color pigmentation, MC seem to require membrane-bound SCF for their migration and survival. The fact that the phenotype was detected in soluble-form SCF transgenic mice (Kunisada *et al*, 1998b) confirmed that only membrane-bound form SCF is necessary for MC survival. In contrast, small numbers of MC survive and migrate to the periphery until 12.5 dpc in Mgf^{Sld}/Mgf^{Sld} mice (Grimm, unpublished observation). In addition, SCF-defective Mgf^{Sl}/Mgf^{Sl} mutant MC development was supported by soluble SCF addition in an *in vitro* culture system (Ito *et al*, 1999). In order to know whether only the membrane-bound form of SCF is required for MC migration and survival in skin tissue, we mated Mgf^{Sld}/Mgf^{Sld} mutant mice with hK14-SCF Tg mice. As this transgenic mutant has both membrane-bound and soluble forms of SCF in the epidermal basal layer, but only the endogenous soluble form of SCF in hair follicles, we expected to observe the functional difference between membrane-bound and soluble-form SCF on MC development between interfollicular epidermal MC and follicular MC. Interestingly, this combination resulted in mice with patchy pigmented skin with unpigmented fur covering the whole body (Yoshida, unpublished observation). There are several possibilities to explain this phenotype. MC may require the membrane-bound form of SCF for their survival, or for their migration from the epidermis to the hair follicle, or for differentiation to produce pigment granules. Each possibility is now under investigation.

CONCLUSIONS

For MC survival and migration, spatiotemporally regulated expression of membrane-bound SCF and signal transduction through c-kit receptor tyrosine kinase is a key event at several stages of MC development. On the other hand, the analysis of its function in MC development also demonstrated the presence of c-kit-independent MC survival, especially at the stem cell stage in postnatal skin. Close investigation of this ligand and receptor signal transduction system will be helpful for understanding common mechanisms in cell survival and migration.

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